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14. ABSTRACT We have identified and characterized a biosensor for auxin and constructed the corresponding biosynthetic cassette. The system is shown in figure 1. Three variants have been made so that we can select on ampicillin, chloramphenicol, or kanamycin. The system also contains a co-transcribed GFP marker for quantitation. After full validation of genotypes we will begin characterizing auxin production and the associated readout provided by the biosensor cassette. Mutagenesis of the host and increased auxin production will follow.					
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Rapid parallel screening for strain optimization

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Reporting Period: 17MAY14 to 16AUG14
Contract No.: HR0011-12-C-0062
Performing Organization: J. Craig Venter Institute
9704 medical Center Drive
Rockville, MD 20850
USA
Principal Investigator: Chuck Merryman

Abstract

We have identified and characterized a biosensor for auxin and constructed the corresponding biosynthetic cassette. The system is shown in figure 1. Three variants have been made so that we can select on ampicillin, chloramphenicol, or kanamycin. The system also contains a co-transcribed GFP marker for quantitation. After full validation of genotypes we will begin characterizing auxin production and the associated readout provided by the biosensor cassette. Mutagenesis of the host and increased auxin production will follow.

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Summary

In total, 108 compounds have been used for enrichment culture and 85 compounds produced colonies (when used as the sole source of carbon and energy). Analysis resulted in the identification of 38 genomes with candidate transcription factors that likely respond to one of the 108 chemicals. Constructs for cloning and evaluating transcription factors were completed previously. An auxin responsive repressor was selected to create the biosensor. The auxin biosynthetic pathway has been installed together with the biosensor. The complete system will now be characterized and we will begin work on improving auxin yields.

Introduction

The overall goal in this contract is to link cell-based production to cell survival and thereby make the engineering of new microbial strains that produce industrially relevant biochemicals routine. Recent synthetic biology techniques can make billions of variant cells. Although, many potentially informative mutants are easily made, product yield can only be determined in a few of these. The majority of industrially relevant biomolecules are not chromophores, naturally discernible, or otherwise easily detected. Nevertheless, genetic circuits are capable of linking chemical production to discernible signals such as growth or color intensity. Such a system would allow numerous mutants and mutant combinations to be examined quickly. Genetic circuits needed to screen mutant populations in parallel rely upon the availability of an appropriate biosensor that activates a reporter gene in a product dependent fashion. These are not routinely available. In this project, genes for two-component and one-component signaling systems (that respond to industrially relevant biomolecules) are identified. To demonstrate that such sensors can be used to maximize product yield, one sensing system will be further engineered. We will reformat this sensor so that it drives expression of a reporter such as an antibiotic resistance marker. This sensor/resistance cassette, and a biosynthetic pathway capable of producing the molecule to which the sensor responds (auxin), will be placed within a heterologous host that does not have an overlapping pathway. Basal synthesis of the targeted chemical (by the orthogonal biosynthetic pathway) activates the sensor and increases transcription of the resistance marker (i.e. reporter). In other words, the fermentation product is also the sensor ligand and thus, biosynthesis drives production of the reporter and a discernable cell phenotype. Targeted, genome-wide and barcoded alterations to the host genome will then be installed. Variants with better and better chemical production are selected by virtue of increased reporter activity.

Methods, Assumptions and Procedures

We have selected an auxin responsive system and constructed the appropriate genetic circuitry both to produce auxin and to simultaneously link that production to a selectable marker and visible marker (Figure 1). The next phase will be ensuring that the biosynthetic pathway is producing auxin and that the chemical is activating the biosensor. Salkowski reagent will be used to determine auxin production. Antibiotic resistance and fluorescence will be used to quantitate the biosensors response. The major assumption at this point is that the level of auxin production

and the dynamic range of the biosensor will overlap. If auxin production is too low to trigger a readout from the biosensor there is no significant issue. Fundamentally, that is the goal of the proposal. Identify which mutations in the host increase auxin production. In comparison, it is possible that auxin biosynthesis from the heterologous cassette will be robust and overwhelm the sensor. In that case, the sensor cassette will be subjected to random mutagenesis to identify a less responsive version that requires more auxin to produce antibiotic resistance.

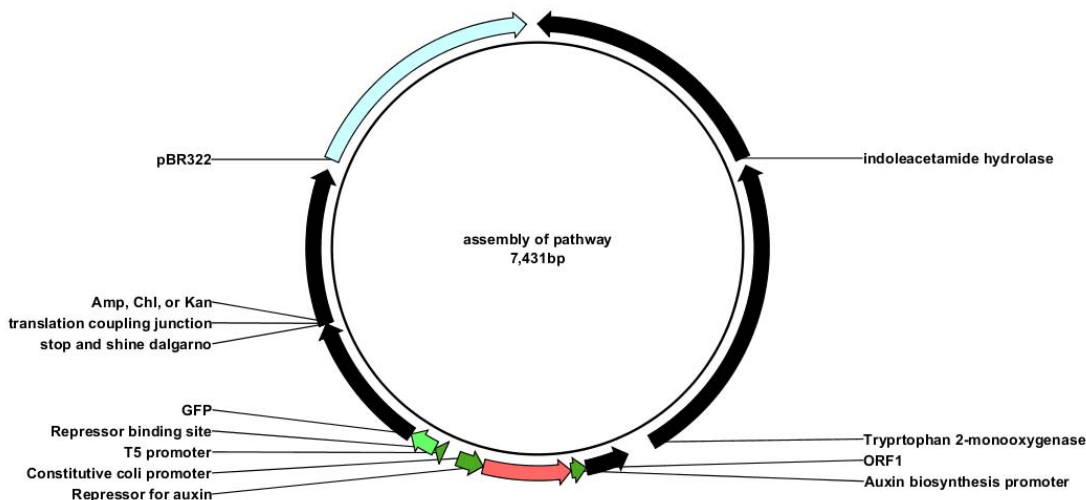


Figure 1. Plasmid construct containing an auxin biosynthesis pathway, and an auxin responsive biosensor controlling the production of GFP and an antibiotic marker. Three different constructs with three different markers were made. Characterization of these systems will reveal if one system has superior performance.

Results and Discussion

Screening of chemicals, processing microbes, construction of necessary plasmids and building of a complete system has been completed. The final system is being characterized. Mutagenesis of the host and increased biosynthesis of auxin will follow.

Conclusions

The results indicate that a chemical made by one organism is likely to be used as food by some other microbe. Bacteria typically utilize the most efficient carbon source available (glucose often being the preferred substrate). More exotic carbon sources are generally subject to catabolite repression and systems for their utilization are activated after preferred carbon sources are exhausted. Besides catabolite repression, sensors are often employed so that the appropriate degradation pathway for a non-preferred carbon source is activated. Our results show that just about any biologically produced chemical will have a corresponding biodegradation pathway in another organism. And, that such biodegradation pathways are often controlled by transcription factors that respond to the presence of the chemical. We have selected one chemical for further study and have combined a biosynthetic pathway from one organism with a transcription factor from a different organism. Altogether, the circuitry shown in figure 1 is designed to both produce auxin and identify which members of a cell population produce the most auxin.

Rapid parallel screening for strain optimization
(HR0011-12-C-0062)

Statement of Work Task List:

- Task 1 (Phase I, Year 1, Months 0-3): Completed (please refer to report HR0011-12-C-2.1)
- Task 2 (Phase I, Year 1, Months 4-9): Completed. Sixty-five isolates have been sequenced.
- Task 3 (Phase I, Year 1, Months 10-12): Completed. Selected microbes have been sequenced, and annotated.
- Task 4 (Phase I, Year 1, months 12): Completed. Ranking of transcription factors for evaluation was delayed by delays in sequencing genomes
- Task 5 (Phase II, Year 2, Months 13-18): Completed. Identification of at least 1 sensor that activates reporter solely in response to the corresponding small molecule.
- Task 6, (Phase II, Year 2, Months 19-24). Completed and beginning. Construction phase is finished and mutagenesis will begin shortly. Generation of sensor/reporter/biosynthetic construct and TRMR-based mutagenesis of host

Planned Activities for the Next Reporting Period

During the next reporting period we will report the results for improving auxin yield via selecting colonies that survive antibiotic selection.

Program Financial Status

In Process & Completed Tasks	Planned Expend	Actual Expend (Cumulative to Date)	% Budget Completion	At Completion	Latest Revised Estimate	Remarks
Task 1	\$59,251	\$59,251	100%	\$59,251	\$59,251	Completed
Task 2	\$69,229	\$69,229	100%	\$69,229	\$69,229	Completed
Task 3	\$124,706	\$124,706	100%	\$124,706	\$124,706	Completed
Task 4	\$255,817	\$255,817	100%	\$255,817	\$255,817	Completed
Task 5	\$255,817	\$255,817	100%	\$255,817	\$255,817	Completed
Task 6	\$199,767	\$50,110.97	N/A	N/A	\$199,767	N/A
Cumulative	\$964,587	\$814,931	84%	N/A	\$964,587	N/A

There is no management reserve or unallocated resources.

Based on the currently authorized work:

- Is current funding sufficient for the current fiscal year? Yes
- Have you included in the report narrative any explanation of the above data and are they cross-referenced? Not applicable; current funding is sufficient for the current fiscal year.